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Synthesis of Omeprazole Analogues and Evaluation of These as Potential Inhibitors of the Multidrug Efflux Pump NorA of *Staphylococcus aureus*▽†

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A series of 11 pyrrolo[1,2-*a*]quinoxaline derivatives, 1a to 1k, sharing structural analogies with omeprazole, a eukaryotic efflux pump inhibitor (EPI) used as an antiulcer agent, was synthesized. Their inhibitory effect was evaluated using *Staphylococcus aureus* strain SA-1199B overexpressing NorA. By determinations of the MIC of norfloxacin in the presence of these EPIs devoid of intrinsic antibacterial activity and used at 128 μg/ml, and by the checkerboard method, compound 1e (MIC decrease, 16-fold; fractional inhibitory concentration index [ΣFIC], 0.18) appeared to be more active than compounds 1b to 1d, reserpine, and omeprazole (MIC decrease, eightfold; ΣFIC, 0.31), followed by compounds 1a and 1f (MIC decrease, fourfold; ΣFIC, 0.37) and 1g to 1k (MIC decrease, twofold; ΣFIC, 0.50 to 0.56). By time-kill curves combining norfloxacin (1/4 MIC) and the most efficient EPIs (128 μg/ml), compound 1e persistently restored the bactericidal activity of norfloxacin (inoculum reduction, 3 $\log_{10}$ CFU/ml at 8 and 24 h), compound 1f led to a delayed but progressive decrease in the number of viable cells, and compounds 1b to 1d and omeprazole acted synergistically (inoculum reduction, 3 $\log_{10}$ CFU/ml at 8 h but further regrowth), while compound 1a and reserpine slightly enhanced norfloxacin activity. The bacterial uptake of norfloxacin monitored by high-performance liquid chromatography confirmed that compounds 1a to 1f increased antibiotic accumulation, as did reserpine and omeprazole. Since these EPIs did not disturb the Δψ and ΔpH, they might directly interact with the pump. A structure-activity relationships study identified the benzimidazole nucleus of omeprazole as the main structural element involved in efflux pump inhibition and highlighted the critical role of the chlorine substituents in the stability and efficiency of compounds 1e to 1f. However, further pharmacomodulation is required to obtain therapeutically applicable derivatives.

In recent years, active efflux has become recognized as a major component of microbial resistance to most classes of antibiotics. This mechanism is mediated by efflux pumps, which are membrane-associated transporters promoting antibiotic extrusion from the cell via an energy-dependent process. Some efflux pumps selectively expel specific antibiotics, while others, referred to as multidrug resistance (MDR) pumps, export a broad array of structurally unrelated compounds (26, 30, 33, 36). MDR is conferred mainly by the efflux systems of the major facilitator superfamily (MFS) of gram-positive bacteria, with the most studied pump being NorA of *Staphylococcus aureus*, while the resistance/nodulation/division efflux systems are the major contributors in gram-negative bacteria (26, 30, 33, 36).

Strategies used to combat efflux-mediated resistance are based on the search for either new antibiotics bypassing the efflux systems or efflux pump inhibitors (EPIs). The latter solution is attractive since a single EPI that is active against MDR pumps and can be used as adjunct therapy should (i) decrease intrinsic resistance, (ii) reverse acquired resistance, and (iii) reduce the emergence of mutants that are highly resistant to a wide range of existing antibiotics (22, 25, 27, 29, 39, 43). Among the candidate EPIs, drugs that are in clinical use for other indications are of interest, since a large amount of data with respect to their pharmacokinetics and toxicity are already available (29). Several of these nonantibiotics have been demonstrated to act as EPIs on NorA and other MDR pumps of gram-positive organisms but at concentrations that are too high to be achieved at therapeutic dosages and/or too toxic. These drugs include reserpine (antihypertensive agent) (1, 9, 16–18, 20, 21, 28, 47), verapamil (antiarrhythmic) (1, 47), omeprazole (antiulcer) (1), paroxetine (antidepressant) (16, 44), and chlorpromazine (neuroleptic) (17). A pharmacomodulation of paroxetine has been conducted to identify the structural features involved in efflux pump inhibition and design new, more potent EPIs (44). Chlorpromazine derivatives have been compared to elucidate the way that they inhibit bacterial efflux pumps (16). However, no such investigations have been performed with omeprazole, although it was the most active bacterial EPI in a previous comparative study (1).

We previously described a novel approach to design pyrrolo[1,2-*a*]quinoxaline derivatives as interesting bioactive ana-

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logues of pyridine, quinoxaline, or quinoline derivatives (11, 12). The aim of this study was to design a series of new pyrrolo[1,2-*a*]quinoxaline compounds mimicking the omeprazole structure and to evaluate their efficiency as EPIs in a model targeting NorA by rationally selected tests. A preliminary structure-activity relationship study allowed us to identify the structural elements of these new omeprazole analogues implicated in EPI activity.

MATERIALS AND METHODS

Chemical syntheses: general procedures for preparation. (i) 2-[{(Pyrrolo[1,2-*a*]quinoxalin-4-yl)methyl}thio]benzimidazoles 6a to 6g, 2-[{(pyrrolo[1,2-*a*]quinoxalin-4-yl)methyl}thio]imidazoles 6h to 6i, 2-[{(pyrrolo[1,2-*a*]quinoxalin-4-yl)methyl}thio]pyridine 6j, and 2-[{(pyrrolo[1,2-*a*]quinoxalin-4-yl)methyl}thio]-7-methoxypyrrolo[1,2-*a*]quinoxaline 6k.

Sodium hydroxide (9.23 mmol) was slowly added over 5 min to a stirred solution of a 2-mercapto derivative (4.62 mmol) in ethanol (30 ml). A solution of 4-chloromethylpyrrolo[1,2-*a*]quinoxaline 5a to 5d (4.62 mmol) in 40 ml of ethanol was slowly added to the 2-mercapto derivative solution at 0°C and stirred for 16 h at room temperature. After the solvent was removed under reduced pressure, the residue was poured into a 2.5% NaOH solution and extracted with chloroform. The organic layer was dried over Na_2SO_4, and concentrated to give compounds 6a to 6k.

(ii) 2-[{(Pyrrolo[1,2-*a*]quinoxalin-4-yl)methyl}sulfinyl]benzimidazoles 1a to 1g, 2-[{(pyrrolo[1,2-*a*]quinoxalin-4-yl)methyl}sulfinyl]imidazoles 1h and 1i, 2-[{(pyrrolo[1,2-*a*]quinoxalin-4-yl)methyl}sulfinyl]pyridine 1j, and 2-[{(pyrrolo[1,2-*a*]quinoxalin-4-yl)methyl}sulfinyl]-7-methoxypyrrolo[1,2-*a*]quinoxaline 1k.

A solution of *m*-chloroperbenzoic acid (2 mmol) in chloroform (20 ml) was added dropwise to ice-cooled solutions of compounds 6a to 6k (2 mmol) in chloroform (35 ml). The reaction mixture was stirred at 0°C for 1 h and then washed with a saturated $NaHCO_3$ solution and dried (Na_2SO_4). After removal of the solvent, the residue was triturated in ethyl acetate, filtered, washed with ethyl acetate, dried, and recrystallized from ethanol to give compounds 1a to 1k (see the supplemental material).

Bacterial strains and media. The wild-type clinical isolate *S. aureus* SA-1199 and its overproducing NorA mutant strain SA-1199B (18, 19, 21) were generously provided as gifts from G. W. Kaatz (University of Michigan). These strains were routinely cultured on Mueller-Hinton (MH) agar (Bio-Rad, Marnes-la-Coquette, France) and broth adjusted to contain 20 μg/ml of Ca^{2+} and 10 μg/ml of Mg^{2+} (AES, Bruz, France) at 37°C. They were stored in 30% glycerol–brain heart broth at −80°C.

Antibiotic and chemicals. Norfloxacin, reserpine, and omeprazole were purchased from Sigma-Aldrich (Saint Quentin Fallavier, France). Solutions of reserpine, omeprazole, and the candidate inhibitors 1a to 1k were extemporaneously prepared in 100% dimethyl sulfoxide (DMSO), but the highest final concentration of DMSO present in all assays (4%, vol/vol) caused no inhibition of bacterial growth (data not shown).

Antibiotic susceptibility tests. Antibiotic susceptibilities of SA-1199, SA-1199B, and the survivors of the time-kill curve experiments were determined by the agar diffusion method (31) using MH medium alone or supplemented with EPIs at 128 μg/ml. Six antibiotics were tested, including all available fluoroquinolone disks, i.e., norfloxacin (5 μg), ciprofloxacin (5 μg), ofloxacin (5 μg), levofloxacin (5 μg), sparfloxacin (5 μg), moxifloxacin (5 μg), and chloramphenicol (30 μg). After overnight incubation at 37°C, the inhibition zone diameters were measured. Results are expressed as the mean values of multiple (at least three) independent experiments.

MICs of norfloxacin and EPIs were determined for SA-1199, SA-1199B, and the survivors of the time-kill experiments by a broth dilution method in tubes under standard conditions (31). Bacterial suspensions were prepared from an MH broth culture obtained after incubation at 37°C in a stirred water bath for 4 to 5 h and further diluted 1/10 in MH broth (≈10^6 CFU/ml). Norfloxacin concentrations ranged between 128 and 0.1 μg/ml, and the EPI concentrations ranged between 512 and 1 μg/ml. In a second series of experiments, antibiotic solutions were combined with each EPI at a final concentration of 128 μg/ml. After an 18-h incubation period at 37°C, the MIC was defined as the lowest concentration that inhibited any visible growth. All tests were done at least in triplicate, and the mode values were retained.

Checkerboard assay. Interactions between norfloxacin and the EPIs on SA-1199B and the survivors of the time-kill experiments were evaluated by a checkerboard titration assay in tubes (7). The bacterial inoculum was prepared as described above for MIC determinations. Norfloxacin was tested at seven concentrations (64 to 1 μg/ml), and each EPI was tested at six concentrations (512 to 16 μg/ml). Rows of tubes containing each drug alone at the same concentrations were also included. Tubes were assessed visually for growth after an 18-h incubation period at 37°C. The effect of drug combinations was estimated at the point of maximal effectiveness by the fractional inhibitory concentration index (ΣFIC), i.e., the sum of the fractional inhibitory concentration of each drug, which in turn is defined as the MIC of each drug when used in combination divided by the MIC of the drug when used alone (7). The ΣFIC data were interpreted according to the following criteria: synergy was defined as an ΣFIC of ≤0.5, addition was defined as $0.5 > \Sigma FIC \leq 1$, indifference was defined as >1 ΣFIC <2, and antagonism was defined as an ΣFIC of ≥2. All experiments were carried out at least three times, and results are expressed as the mode values.

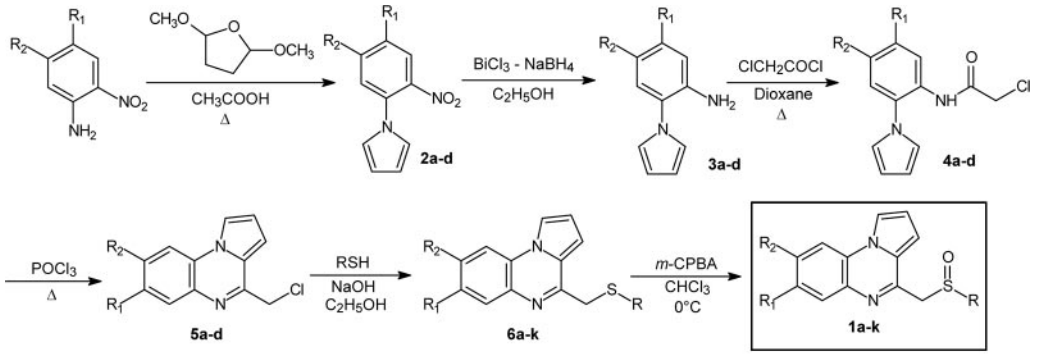
Time-kill curves. Interactions between norfloxacin and the most efficient EPIs or the reference EPIs on SA-1199B were also evaluated by the time-kill curve method (7). An initial bacterial inoculum of 10^6 CFU/ml in the logarithmic phase was prepared as described above for MIC determinations. The final concentrations were 16 μg/ml of norfloxacin and 128 μg/ml of the EPIs. Surviving cells were enumerated by dilution plating in triplicate onto MH agar at 0 (inoculum control), 2, 4, 8, and 24 h of incubation at 37°C. Results are expressed as the log percentage of survival and represent the mean values of three different experiments. The limit of sensitivity was set at 1 $\log_{10}$ CFU/ml to avoid any carryover effect.

Norfloxacin and omeprazole assays. For the uptake experiments, bacterial cultures of SA-1199 and SA-1199B were grown to mid-exponential phase in 300 ml of MH broth in a shaken water bath for 4 to 5 h at 37°C. Cells were centrifuged, pelleted, washed, and resuspended in sodium phosphate buffer (50 mM) at pH 7.0. The suspension was then divided into 0.2-ml aliquots, and bacteria were enumerated by dilution plating as indicated above. Each suspension was incubated with norfloxacin at one-quarter the MIC alone or in combination with each EPI at 128 μg/ml at time zero (T_0). After 5, 10, 15, and 20 min, cells were separated from the extracellular medium by differential centrifugation through a water-impermeable silicone-paraffin oil barrier (1.029 g/cm^3) and directly lysed by the orthophosphoric acid present in the bottom of the tube. The amounts of norfloxacin in the lysate were determined by high-performance liquid chromatography (HPLC) with a Supercosil C_{18} column (Waters, Guyancourt, France). The mobile phase consisted of 80% phosphate buffer (H_3PO_4-KH_2PO_4 [10 mM] at pH 2.5) and 20% acetonitrile. The excitation and emission wavelengths were 240 and 280 nm, respectively. Protein concentrations were determined with a commercially available kit based on the use of bicinchoninic acid and bovine serum albumin as standards (Pierce, IL). Results were expressed as ng drug accumulated/mg cell protein. Experiments were done in triplicate, and results given are the means of the three experiments. The norfloxacin and omeprazole concentrations were also determined during the time-kill curve experiments in the supernatants at time zero T_0 and at 24 h (T_{24}) by HPLC using the conditions indicated above for the antibiotic. For omeprazole and compounds 1a to 1f, the mobile phase consisted of 65% phosphate buffer (50 mM Na_2HPO_4, pH 6.5) and 35% acetonitrile. The wavelength of detection was 302 nm for omeprazole (48), 280 nm for compounds 1a to 1d, and 300 nm for compounds 1e to 1f.

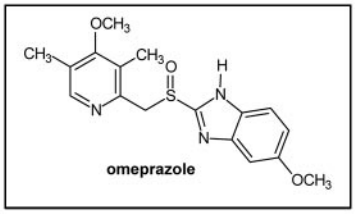
Δψ and ΔpH determinations. Accumulation of the membrane-permeant cation tetraphenylphosphonium and salicylic acid was used to measure the transmembrane electrical potential, Δψ, and the ΔpH, respectively, in the absence or presence of reserpine (33 μM), carbonyl cyanide *m*-chlorophenylhydrazone (CCCP) (100 μM), omeprazole, or the new compounds 1a to 1f (128 μg/ml). After incubation in a shaken bath for 4 h at 37°C, MH broth cultures were divided into aliquots that were placed in ice. The protein content of one aliquot was determined as indicated above. These samples were then warmed for 5 min to room temperature. To measure the Δψ, cells were labeled with 1 μM [^{3}H]tetraphenylphosphonium (29 Ci/mmol; Amersham Biosciences Corp., Buckinghamshire, United Kingdom) for 10 min in the presence or absence of the EPIs. One aliquot was treated with 4% DMSO (vol/vol) for 10 min prior to labeling in order to obtain a background value. For the ΔpH, EPIs were added to samples for 20 min. Cells were then labeled with 20 μM [^{14}C]salicylic acid (47 mCi/mmol; Perkin-Elmer, Wellesley, MA). For both experiments, samples were centrifuged for 10 min at 4°C and 13,000 × *g*, and the radioactivity present in both phases (cells and supernatant) was measured using a scintillation counter. The Δψ and ΔpH were calculated by using a cell volume of 4.2 μl/mg of cell protein as previously described (17, 38). The results of these experiments, repeated three times, were expressed as the mean values ± standard deviations.

RESULTS

Chemistry. A series of new pyrrolo[1,2-*a*]quinoxaline derivatives, compounds 1a to 1k (Fig. 1), has been synthesized by



Compounds	R_1	R_2
2a, **3a**, **4a**, **5a**	H-	H-
2b, **3b**, **4b**, **5b**	CH_3O-	H-
2c, **3c**, **4c**, **5c**	Cl-	H-
2d, **3d**, **4d**, **5d**	H-	Cl-



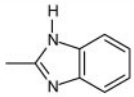
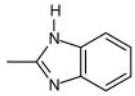
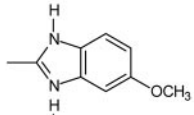
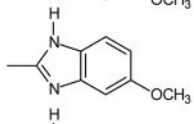
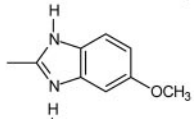
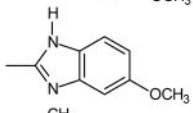
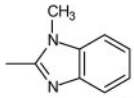
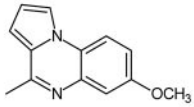
Compounds	R	R_1	R_2
6a, **1a**		H -	H -
6b, **1b**		CH_3O -	H -
6c, **1c**		H -	H -
6d, **1d**		CH_3O -	H -
6e, **1e**		Cl -	H -
6f, **1f**		H -	Cl -
6g, **1g**		H -	H -
6h, **1h**		H -	H -
6i, **1i**		H -	H -
6j, **1j**		H -	H -
6k, **1k**		H -	H -

FIG. 1. Synthesis of pyrrolo[1,2-*a*]quinoxaline derivatives 1a to 1k, new structural analogues of omeprazole.

the pharmacomodulation of a basic heterocyclic synthon related to the structure of omeprazole. This synthesis has been achieved by starting from various substituted 2-nitro-anilines in six steps. The Clauson-Kaas reaction (8) of anilines with 2,5-dimethoxytetrahydrofuran in acetic acid gave the pyrrolic derivatives 2a to 2d (12), which were reduced using $BiCl_3$-$NaBH_4$ treatment to provide the expected 1-(2-aminophenyl)pyrroles 3a to 3d (37). The reaction of chloroacetyl chloride with 3a to 3d (12) led to chloracetamides 4a to 4d (10). The 4-chloromethylpyrrolo[1,2-*a*]quinoxalines 5a to 5d were prepared by

TABLE 1. In vitro activities of reserpine, omeprazole, and the pyrrolo[1,2-*a*]quinoxaline derivatives 1a to 1k as EPIs by the disk diffusion method for SA-1199B[a]

Strain	EPI	EPI concn (μg/ml)	Inhibition zone diameter (mm)						
			Nor	Cip	Ofx	Lvx	Spx	Mxf	C
SA-1199			27	26	27	30	30	31	27
SA-1199B			6	13	18	21	27	27	24
	Res	128	13	22	21	24	28	28	25
		64	12	20	20	24	27	28	25
		32	12	18	19	24	27	28	24
		16	9	16	19	22	27	28	24
	Om	128	14	22	23	25	28	30	25
		64	12	19	20	23	27	29	24
		32	6	15	18	21	27	27	24
		16	6	13	18	21	26	27	24
	1a	128	10	19	23	24	28	28	25
	1b	128	13	22	25	24	28	28	26
	1c	128	15	24	25	24	27	28	26
	1d	128	16	24	25	24	29	28	27
	1e	128	18	25	26	27	28	28	27
	1f	128	10	19	21	23	27	27	25
	1g	128	6	15	20	23	27	27	25
	1h	128	6	15	19	22	27	27	25
	1i	128	6	14	19	22	27	27	25
	1j	128	6	17	20	22	27	27	25
	1k	128	6	17	19	22	27	27	25

[a] Abbreviations: Res, reserpine; Om, omeprazole; Nor, norfloxacin; Cip, ciprofloxacin; Ofx, ofloxacin; Lvx, levofloxacin; Spx, sparfloxacin; Mxf, moxifloxacin; C, chloramphenicol.

the cyclization of these amides, 4a to 4d, in refluxing phosphorus oxychloride according to the Bischler-Napieralski reaction procedure (3, 45). The 4-chloromethylpyrrolo[1,2-*a*]quinoxalines 5a to 5d (10) were then condensed with various substituted 2-mercapto derivatives in ethanol in the presence of sodium hydroxide to give the corresponding sulfides 6a to 6k (6, 15). The sulfoxides 1a to 1k were obtained by low-temperature oxidation of sulfides 6a to 6k with *m*-chloroperbenzoic acid in chloroform (4, 23).

Antibiotic and EPI susceptibilities of the *S. aureus* strains. A strategy including four steps has been designed to investigate the efficiency of new EPIs in a model targeting NorA and using SA-1199 and SA-1199B. In preliminary experiments, the MICs of the reference and putative EPIs were determined in order to subsequently use them at concentrations devoid of bacterial toxicity and hence to evaluate their efficiency only as EPIs. Reserpine, omeprazole, and pyrrolo[1,2-*a*]quinoxaline derivatives 1a to 1k were found to lack any intrinsic antibacterial activity (MIC > 512 μg/ml).

In a first step of screening, the high-throughput disk diffusion method was used to characterize the profiles of the test strains, to identify the preferential substrate and the optimal concentration of the reference EPIs, and to detect any EPI activity of the tested compounds. SA-1199B exhibited reduced inhibition zones towards fluoroquinolones, particularly norfloxacin and ciprofloxacin, and a slightly decreased inhibition zone towards chloramphenicol compared to SA-1199 (Table 1). At concentrations less than 32 μg/ml, omeprazole had no effect on norfloxacin and ciprofloxacin activity, while at 128 μg/ml, it was evident. Thus, the latter concentration was subsequently used for all tested EPIs in order to maximize the chance of observing an effect. Actually, at 128 μg/ml, all new synthesized compounds increased the inhibition zones of the fluoroquinolones, particularly those of the most affected drugs, norfloxacin and ciprofloxacin, and thus acted as EPIs. In a second step of screening, these data were confirmed by MIC determinations. *S. aureus* strain SA-1199B exhibited norfloxacin MICs that were 32 times higher than those for SA-1199, and the presence of each EPI at 128 μg/ml resulted in a reduction in the norfloxacin MIC (2- to 16-fold) for SA-1199B and, to a much lesser degree, for SA-1199 (Table 2). Compound 1e appeared to be the most active, as determined by the disk diffusion method.

Effect of norfloxacin and EPI combinations by the checkerboard method. In a third step of screening, the combinations between the preferential substrate and the EPIs were investigated by both reference methods. The checkerboard method aims at evaluating the bacteriostatic effect of antibiotic combinations according to their concentrations at a fixed time of 18 h (7). This method was applied to the combination of norfloxacin with reserpine, omeprazole, and each new compound, compounds 1a to 1k, on SA-1199B, and the interpretation was adapted to antibiotic-EPI combinations by calculating the ΣFIC with the MIC of these atoxic EPIs taken as 1,024 μg/ml, i.e., the value that was twice the maximal concentration tested. By this mean, reserpine, omeprazole, and the five candidate inhibitors 1a to 1f appeared to be synergistic on SA-1199B, while the other new compounds, compounds 1g to 1k, were only additive. The rank order of decreasing activity of the EPIs was identical to that found by MIC determinations, i.e., compound 1e was more active than reserpine, omeprazole, and

TABLE 2. In vitro activities of reserpine, omeprazole, and the pyrrolo[1,2-*a*]quinoxaline derivatives 1a to 1k as EPIs by norfloxacin MIC determinations for SA-1199 and SA-1199B and by the checkerboard method for SA-1199B[a]

Strain	Nor MIC (μg/ml)	EPI concn (μg/ml)	MIC (μg/ml) of Nor + EPI:												
			Res	Om	1a	1b	1c	1d	1e	1f	1g	1h	1i	1j	1k
SA-1199	2	128	1	1	1	1	1	1	1	1	2	2	2	2	2
SA-1199B	64	128	8	8	16	8	8	8	4	16	32	32	32	32	32
Optimal concn (μg/ml)[b]			16/32	16/64	16/128	16/64	16/64	16/64	8/64	16/128	32/64	32/64	32/64	32/64	16/256
ΣFIC			0.31	0.31	0.37	0.31	0.31	0.31	0.18	0.37	0.56	0.56	0.56	0.56	0.5

[a] Abbreviations: Nor, norfloxacin; Res, reserpine; Om, omeprazole.
[b] Optimal norfloxacin/EPI concentrations by the checkerboard method, at which the ΣFIC was calculated.

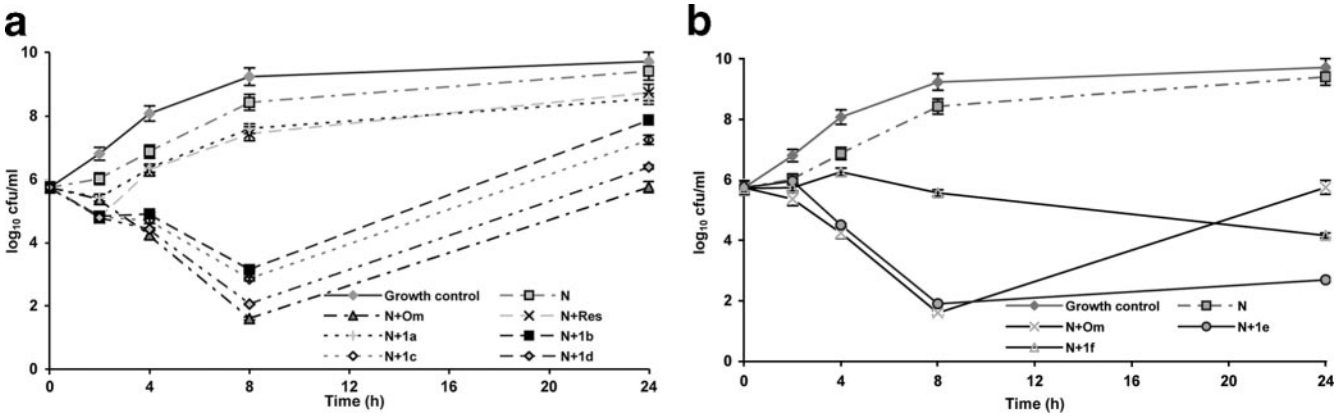


FIG. 2. Time-kill curves of SA-1199B for norfloxacin alone or combined with the EPIs. Norfloxacin was used at one-quarter the MIC (16 μg/ml) alone (N) or combined with omeprazole (N+Om) and (a) reserpine (N+Res) or new derivatives 1a to 1d (N+1a-d) and (b) 1e and 1f (N+1e-f) at 128 μg/ml.

compounds 1b to 1d, followed by compounds 1a, 1f, and 1g to 1k (Table 2).

Effect of norfloxacin and EPI combinations by the time-kill curve method. The objective of the time-kill curve method is to evaluate the bactericidal effect of antibiotic combinations according to the time at some selected concentrations (7). This method was applied to the combination of norfloxacin (one-quarter the MIC, i.e., 16 μg/ml) with the reference EPIs, omeprazole and reserpine, and the most active compounds, compounds 1a to 1f, at 128 μg/ml, on SA-1199B. Norfloxacin (Fig. 2) or the EPIs alone (data not shown) at the concentrations used did not significantly inhibit the growth of the test organism (difference ≤ 1 $\log_{10}$ CFU/ml). According to the usual definition, all tested combinations appeared to be synergistic by this method since they decreased the bacterial growth significantly more than either norfloxacin or each EPI alone. Indeed, the combination of norfloxacin with the reference EPIs showed that reserpine, after a short initial decrease in viable cells, hardly enhanced the norfloxacin activity. In contrast, omeprazole produced a 4-$\log_{10}$ decrease of the starting inoculum at 8 h, followed by a regrowth, leading to a bacterial count similar to that of the inoculum at 24 h. Compound 1a behaved similarly to reserpine. Compounds 1b to 1d gave curves similar to those for omeprazole but were less active in reversing norfloxacin activity, particularly at 24 h. Finally, compound 1f led to a delayed but progressive decrease in viable cells, while compound 1e demonstrated the strongest activity, yielding to a deeper decrease in the initial inoculum at 8 h and persistently restoring the bactericidal activity of norfloxacin (Fig. 2b). Surviving cells recovered in each experiment were tested for their antibiotic and/or EPI susceptibilities. They exhibited the same disk diffusion pattern as SA-1199B and gave the same norfloxacin MIC (64 μg/ml) and the same ΣFIC (0.18) by the checkerboard method as SA-1199B, simultaneously used as control. Thus, survivors were not mutants with increased resistance to norfloxacin or EPI inhibition. The HPLC assay showed that during the time kill curve experiments, norfloxacin concentrations were identical at T_0 (16 ± 0.8 μg/ml) and T_{24} (16 ± 1.5 μg/ml). In contrast, the EPI concentrations considerably decreased from T_0 (128 ± 10 μg/ml) to T_{24} for omeprazole (40 ± 8 μg/ml), compounds 1b to 1d (54 ± 8 μg/ml), and compound 1a (72 ± 9 μg/ml), while compounds 1e and 1f appeared to be more stable (98 ± 9 μg/ml at T_{24}). Consequently, the inhibitory effect of the EPIs in time-kill experiments partially paralleled their stability.

Effect of EPIs on norfloxacin bacterial uptake. Finally, a fourth step ensured that the previously demonstrated synergistic effect was actually related to the increase in the antibiotic substrate accumulation by the putative EPIs. The intrabacterial accumulation of norfloxacin at 16 μg/ml alone or combined with the reference and the most efficient EPIs at 128 μg/ml was determined by HPLC. In SA-1199 (Fig. 3a), the accumulation of norfloxacin reached near equilibrium at 5 min and thereafter remained at the same level up to 20 min, at around 400 to 450 ng/mg cell protein. The addition of reserpine, omeprazole, and compounds 1a to 1f barely increased the final level of norfloxacin accumulation (10%). In SA-1199B (Fig. 3b), the accumulation of norfloxacin was significantly lower than that observed in SA-1199, reaching 235 ng/mg cell protein at 5 min and progressively up to 350 ng/mg cell protein at 20 min. The addition of all EPIs substantially increased the norfloxacin accumulation in SA-1199B, up to 270 to 315 ng/mg cell protein at 5 min and around 400 to 450 ng/mg cell protein at 20 min, increasing by 40% of the final level of norfloxacin accumulation. Thus, the synergistic effect demonstrated by the EPIs was correlated with the partial restoration of norfloxacin uptake.

Effect of the inhibitors on the Δψ and ΔpH. In order to investigate the basis of the EPI effect of omeprazole and related compounds on NorA, the Δψ and ΔpH of the cells were determined for SA-1199B, alone and in the presence of these drugs, compared to reserpine and CCCP, used as controls. The Δψ of SA-1199B cells in MH broth was −126 mV ± 6 mV. In the presence of reserpine or omeprazole, this value was slightly lowered, to −140 ± 10 mV and −140 ± 6 mV, respectively, while in the presence of the uncoupler agent CCCP, it became null. Compounds 1a to 1f had activity similar to that of omeprazole, leading to a Δψ of −130 ± 10 mV. The ΔpH of SA-1199B in MH was +0.55 ± 0.05 over the entire 20-min time course of the experiment. The addition of reserpine, omeprazole, or compounds 1a to 1f had no effect (+0.55 ± 0.05). In contrast, the ΔpH was abolished by CCCP (ΔpH = 0 ± 0.1). Thus, the putative

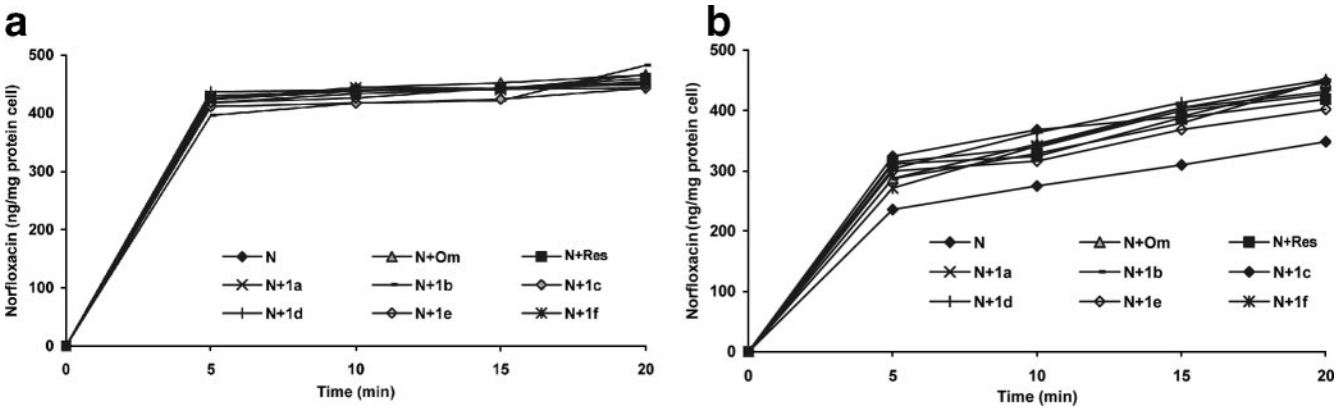


FIG. 3. Intracellular accumulation of norfloxacin alone or combined with EPIs. Norfloxacin was used at one-quarter the MIC (16 μg/ml) alone (N) or combined with omeprazole (N+Om) and (a) reserpine (N+Res) or new derivatives 1a to 1d (N+1a-d) and (b) 1e and 1f (N + 1e-f) at 128 μg/ml. Intracellular accumulation of norfloxacin in (a) SA-1199 and (b) SA-1199B is shown.

EPIs did not significantly influence the electrical potential and the *trans*-membrane pH gradient of the bacterial cells, at least at the concentrations used.

DISCUSSION

In this study, a series of 11 new pyrrolo[1,2-*a*]quinoxaline derivatives, compounds 1a to 1k, was synthesized from commercially available nitroanilines and developed as a template for the design of new bioisosters of omeprazole by various pharmacomodulations in order to obtain new compounds endowed with a higher activity as bacterial EPIs. These derivatives were then evaluated in a model targeting the NorA efflux pump of *S. aureus* (18–21, 28, 42, 47). Indeed, although NorA modestly contributes to wild-type fluoroquinolone resistance, first-step mutants, by diminishing the intracellular drug concentrations, tend to subsequently accumulate additional target mutations under treatment, leading to the commonly encountered high-level fluoroquinolone-resistant clinical strains (2). Moreover, NorA is the prototype of other MFS pumps with 12 transmembrane segments, such as PmrA in *Streptococcus pneumoniae* (35), and has generally served as the model for studying EPIs of MDR pumps in gram-positive organisms (22). EPIs that are active on NorA are hence expected to inhibit at least some of the related pumps.

The model used to assess the potential EPI activity of the 11 new pyrrolo[1,2-*a*]quinoxaline derivatives, compounds 1a to 1k, was based on the isogenic strains SA-1199 and SA-1199B, classically used to investigate NorA inhibition. Our results regarding the susceptibilities of these strains to norfloxacin and the reference EPIs by MIC determinations (1, 9, 16, 17, 19–21, 32, 42), time-kill curves (1), and accumulation assays (16, 42, 46) were in agreement with the literature. The antibiotic resistance and inhibition profile of SA-1199B reflected the overexpression of NorA, a pump that preferentially exports the hydrophilic fluoroquinolones pefloxacin and ciprofloxacin rather than the more hydrophobic drugs of this class, sparfloxacin and moxifloxacin, and has a limited effect on the neutral chloramphenicol (18–21, 28, 42, 47). The slight potentiation of norfloxacin by the EPIs on wild-type strain SA-1199 is related to the low-level expression of NorA in wild-type strains of *S. aureus* (1, 20–22, 32). However, SA-1199B exhibited high-level fluoroquinolone resistance that was not completely reversed by the EPIs, as particularly apparent in norfloxacin accumulation experiments. In fact, SA-1199B is a double-target (A116E in GrlA)/efflux mutant (21). A lesser affinity of the drug for its modified target might lead to a decreased accumulation into the bacterial cell that cannot be amended by EPIs. Accordingly, we observed a total restoration of the norfloxacin susceptibility by reserpine, using MIC determinations, for a single NorA mutant (SA-1) selected in our laboratory (2). Although SA-1199B has been used mostly to investigate the EPI activity on NorA, allowing comparisons to data reported in the literature, a single NorA mutant such as SA-1 might be more convenient for further studies.

By this strategy, all novel derivatives appeared to have EPI properties, and one compound (compound 1e) was consistently more active than omeprazole. Indeed, compound 1e at 128 μg/ml reduced the norfloxacin MIC for SA-1199B by 16-fold. The "minimum potentiating concentration" required to enhance the activity of the preferential substrate against a test strain by at least eightfold (22), which was also the "minimal effective concentration" of EPI that produced the maximal reduction in the substrate MIC (32), was 64 μg/ml, and the lowest active concentration (i.e., capable of reducing the norfloxacin MIC by twofold) was 32 μg/ml. At concentrations ≥256 μg/ml, compound 1e shifted the norfloxacin MIC to 4 μg/ml. Other commercially available drugs such as verapamil paroxetine, chlorpromazine, and their derivatives (1, 47), the novel P-gp inhibitors currently being developed for the treatment of drug resistance tumors (9, 22, 32), and EPIs selected by the screening of plant extracts (34) exhibited similar potentiating effects on NorA. The screening of a chemical library and the further synthesis of indole derivatives have yielded inhibitors of NorA with a much higher potency (28, 41), but their clinical safety has not been established. However, although proton pump inhibitors are well-tolerated, maximal serum concentrations following usual doses are well below the concentrations at which compound 1e acted as an EPI (5, 14).

This limited series of 11 pyrrolo[1,2-*a*]quinoxaline derivatives, designed as omeprazole analogues, has been synthesized in order to initiate a preliminary structure-activity relationship

study, allowing further pharmacomodulations. Biological results highlighted the importance of the benzimidazole moiety (compounds 1a to 1f), since its replacement by an imidazole (compounds 1h and 1i), a pyridine (compound 1j), or a pyrrolo[1,2-*a*]quinoxaline ring (compound 1k) resulted in a significant loss of potency. Moreover, methoxy-substituted compounds 1b to 1f, whatever the substitution position, on either the benzimidazole or the pyrrolo[1,2-*a*]quinoxaline nucleus, were more potent that the unsubstituted compound 1a. The introduction of a second methoxy substituent (compound 1d) was not beneficial compared to the monosubstituted derivatives 1b and 1c. Otherwise, the introduction of one chlorine atom at position 7 or 8 of the pyrroloquinoxaline heterocycle provided an efficient restoration of the bactericidal activity of norfloxacin (compounds 1e and 1f compared to compound 1c). Nevertheless, the 7-chloro-substituted compound 1e was more active than its 8-cholor-substituted homologue, compound 1f. By reference to the omeprazole chemical structure, the replacement of the pyridine ring by a pyrrolo[1,2-*a*]quinoxaline nucleus (compounds 1a to 1f) did not substantially change the EPI activity. However, whatever the other structural modifications were, omeprazole and pyrrolo[1,2-*a*]quinoxaline analogues 1a to 1f acted as EPIs on the NorA efflux pump only at high concentrations (64 to 128 μg/ml). Finally, the *N*-methylated compound 1g, compared to compound 1a, was devoid of EPI activity, suggesting the importance of the amino benzimidazolyl proton in the EPI activity.

This structure-activity relationship study might also contribute to analyses of the mode of action of omeprazole and its analogues as bacterial EPIs. Indeed, the inhibition of MFS pumps in gram-positive organisms, such as the single-component system NorA, can be due either to the disturbance of the source of energy or to a direct interaction with the pump by steric obstruction or substrate competition. Compounds 1a to 1f, as omeprazole and reserpine (17), did not significantly modify the electrical potential and the *trans*-membrane pH gradient, in contrast to CCCP (17), and thus did not seem to alter the source of energy of this proton-dependent pump. Hence, these EPIs might interact directly with NorA. However, a mode of action analogous to that of omeprazole with the eukaryotic H^+/K^+ pump is unlikely, since the pH is not favorable for the activation of our compounds into a reactive sulfenamide (13, 14). Cloning of the *norA* gene and random mutagenesis followed by the selection of isolates resistant to the combination of norfloxacin and omeprazole, as previously performed for Bmr and reserpine in *Bacillus subtilis* and reserpine (24), would be helpful to elucidate the mode of action of these EPIs.

Furthermore, time-kill data showed a late regrowth in the presence of subinhibitory concentrations of norfloxacin with omeprazole or its analogues 1a to 1d. This phenomenon has already been reported for reserpine and omeprazole without a definite explanation (1). In this study, we have demonstrated that these survivors were not mutants with enhanced norfloxacin resistance or reduced susceptibility to EPI inhibition and that the antibiotic was stable over the time-kill experiment period. In contrast, omeprazole and the related compounds underwent a substantial albeit variable degradation. The regrowth observed after 8 h with compounds 1b to 1d, in contrast to compounds 1e and 1f, might be related to a greater instability of the nonchlorinated compounds than the chlorinated ones. It also might be due to the reversible versus irreversible binding of these EPIs to the lipophilic binding pocket of NorA: EPI removal should allow the pump to function de novo, conferring norfloxacin resistance, thus permitting the bacterial multiplication to start again. Indeed, such reversible binding has been observed for omeprazole and the H^+/K^+-ATPase (40). Finally, one cannot exclude that compounds 1a to 1d transiently induced efflux pumps other than NorA, in contrast with compounds 1e and 1f.

In conclusion, a series of new omeprazole analogues has been synthesized and evaluated by a cost-effective and time-saving general strategy. Concordant results showed that all novel derivatives had EPI properties, and one compound (compound 1e) was more active than omeprazole, particularly in restoring the bactericidal activity of norfloxacin over a prolonged period. A structure-activity relationship study identified the benzimidazole nucleus as the key element of efflux pump inhibition and the chlorine at position 7 of the pyrroloquinoxaline heterocycle as a critical substitution. However, further pharmacomodulation is required to obtain therapeutically appropriate EPIs.

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REFERENCES

1. **Aeschlimann, J. R., L. D. Dresser, G. W. Kaatz, and M. J. Rybak.** 1999. Effects of NorA inhibitors on in vitro antibacterial activities and postantibiotic effects of levofloxacin, ciprofloxacin, and norfloxacin in genetically related strains of *Staphylococcus aureus*. Antimicrob. Agents Chemother. **43:** 335–340.
2. **Ba, B. B., C. Arpin, C. Vidaillac, A. Chausse, M. C. Saux, and C. Quentin.** 2006. Activity of gatifloxacin in an in vitro pharmacokinetic-pharmacodynamic model against *Staphylococcus aureus* strains susceptible to ciprofloxacin or exhibiting various levels and mechanisms of ciprofloxacin resistance. Antimicrob. Agents Chemother. **50:**1931–1936.
3. **Bischler, A., and B. Napieralski.** 1893. Zur Kenntnis einer neuen Isochinolinsynthese. Ber. Dtsch. Chem. **26:**1903–1908.
4. **Cereda, E., M. Turconi, A. Ezhaya, A. Brambilla, F. Pagani, and A. Donetti.** 1987. Anti-secretory and anti-ulcer activities of some new 2-(2-pyridylmethyl-sulfinyl)-benzimidazoles. Eur. J. Med. Chem. **22:**527–535.
5. **Ching, M. S., G. W. Mihaly, P. W. Angus, D. J. Morgan, S. Devenish-Meares, and N. D. Yeomans.** 1991. Oral bioavailability of omeprazole before and after chronic therapy in patients with duodenal ulcer. Br. J. Clin. Pharmacol. **31:**166–170.
6. **Cho, S. Y., S. K. Kang, S. S. Kim, H. G. Cheon, J. K. Choi, and E. K. Yum.** 2001. Synthesis and SAR of benzimidazole derivatives containing oxycyclic pyridine as gastric H^+/K^+-ATPase inhibitors. Bull. Korean Chem. Soc. **22:** 1217–1223.
7. **Eliopoulos., G. M.** 1996. Antimicrobial combinations, p. 330–396. *In* V. Lorian (ed.), Antibiotics in laboratory medicine. Williams & Wilkins, Baltimore, MD.
8. **Elming, N., and N. Clauson-Kaas.** 1952. The preparation of pyrroles from furans. Acta Chem. Scand. **6:**867–874.
9. **Gibbons, S., M. Oluwatuyi, and G. W. Kaatz.** 2003. A novel inhibitor of multidrug efflux pumps in *Staphylococcus aureus*. J. Antimicrob. Chemother. **51:**13–17.
10. **Guillon, J., B. Pfeiffer, P. Renard, D. Manchez, A. Kervran, and S. Rault.** 1998. Synthesis of new pyrrolo[1,2-*a*]quinoxalines: potential non peptide glucagon receptor antagonist. Eur. J. Med. Chem. **33:**293–308.
11. **Guillon, J., M. Boulouard, V. Lisowski, S. Stiebing, V. Lelong, P. Dallemagne, and S. Rault.** 2000. Synthesis of new 2-(aminomethyl)-4-phenylpyrrolo[1,2-*a*]-quinoxalines and their preliminary in-vivo central dopamine antagonist activity evaluation in mice. J. Pharm. Pharmacol. **52:**1369–1375.
12. **Guillon, J., P. Grellier, M. Labaied, P. Sonnet, J. M. Leger, R. Deprez-**

Poulain, I. Forfar-Bares, P. Dallemagne, N. Lemaitre, F. Pehourcq, J. Rochette, C. Sergheraert, and C. Jarry. 2004. Synthesis, antimalarial activity, and molecular modeling of new pyrrolo[1,2-*a*]quinoxalines, bispyrrolo[1,2-*a*]quinoxalines, bispyrido[3,2-*e*]pyrrolo[1,2-*a*]pyrazines, and bispyrrolo[1,2-*a*]thieno[3,2-*e*]pyrazines. J. Med. Chem. **47:**1997–2009.

13. **Hellstrom, P. M., and S. Vitols.** 2004. The choice of proton pump inhibitor: does it matter? Basic Clin. Pharmacol. Toxicol. **94:**106–111.
14. **Horn, J.** 2000. The proton-pump inhibitors: similarities and differences. Clin. Ther. **22:**266–280.
15. **If, R. J., C. A. Dyke, D. J. Keeling, E. Meenan, M. L. Meeson, M. E. Parsons, C. A. Price, C. J. Theobald, and A. H. Underwood.** 1989. 2-[[(4-Amino-2-pyridyl)methyl]sulfinyl]benzimidazole H^+/K^+-ATPase inhibitors: the relationship between pyridine basicity, stability and activity. J. Med. Chem. **32:**1970–1977.
16. **Kaatz, G. W., V. V. Moudgal, S. M. Seo, J. B. Hansen, and J. E. Kristiansen.** 2003. Phenylpiperidine selective serotonin reuptake inhibitors interfere with multidrug efflux pump activity in *Staphylococcus aureus*. Int. J. Antimicrob. Agents **22:**254–261.
17. **Kaatz, G. W., V. V. Moudgal, S. M. Seo, and J. E. Kristiansen.** 2003. Phenothiazines and thioxanthenes inhibit multidrug efflux pump activity in *Staphylococcus aureus*. Antimicrob. Agents Chemother. **47:**719–726.
18. **Kaatz, G. W., S. M. Seo, and T. J. Foster.** 1999. Introduction of a *norA* promoter region mutation into the chromosome of a fluoroquinolone-susceptible strain of *Staphylococcus aureus* using plasmid integration. Antimicrob. Agents Chemother. **43:**2222–2224.
19. **Kaatz, G. W., S. M. Seo, and C. A. Ruble.** 1993. Efflux-mediated fluoroquinolone resistance in *Staphylococcus aureus*. Antimicrob. Agents Chemother. **37:**1086–1094.
20. **Kaatz, G. W., and S. M. Seo.** 1995. Inducible NorA-mediated multidrug resistance in *Staphylococcus aureus*. Antimicrob. Agents Chemother. **39:**2650–2655.
21. **Kaatz, G. W., and S. M. Seo.** 1997. Mechanisms of fluoroquinolone resistance in genetically related strains of *Staphylococcus aureus*. Antimicrob. Agents Chemother. **41:**2733–2737.
22. **Kaatz, G. W.** 2005. Bacterial efflux pump inhibition. Curr. Opin. Investig. Drugs **6:**191–198.
23. **Katsuaki, K., K. Oda, T. Kaneko, H. Satoh, and A. Nohara.** 1990. Synthesis of 2-[[(4-fluoroalkoxy-2-pyridyl)methyl]sulfinyl]-*1H*-benzimidazoles as antiulcer agents. Chem. Pharm. Bull. **38:**2853–2858.
24. **Klyachko, K. A., S. Schuldiner, and A. A. Neyfakh.** 1997. Mutations affecting substrate specificity of the *Bacillus subtilis* multidrug transporter Bmr. J. Bacteriol. **179:**2189–2193.
25. **Lewis, K.** 2001. In search of natural substrates and inhibitors of MDR pumps. J. Mol. Microbiol. Biotechnol. **3:**247–254.
26. **Li, X. Z., and H. Nikaido.** 2004. Efflux-mediated drug resistance in bacteria. Drugs **64:**159–204.
27. **Lomovskaya, O., and W. J. Watkins.** 2001. Efflux pumps: their role in antibacterial drug discovery. Curr. Med. Chem. **8:**1699–1711.
28. **Markham, P. N., E. Westhaus, K. Klyachko, M. E. Johnson, and A. A. Neyfakh.** 1999. Multiple novel inhibitors of the NorA multidrug transporter of *Staphylococcus aureus*. Antimicrob. Agents Chemother. **43:**2404–2408.
29. **Marquez, B.** 2005. Bacterial efflux systems and efflux pumps inhibitors. Biochimie **87:**117–147.
30. **McKeegan, K. S., M. I. Borges-Walmsley, and A. R. Walmsley.** 2004. Structural understanding of efflux-mediated drug resistance: potential routes to efflux inhibition. Curr. Opin. Pharmacol. **4:**479–486.
31. **Members of the SFM Antibiogram Committee.** 2003. Comité de l'Antibiogramme de la Société Française de Microbiologie Report 2003. Int. J. Antimicrob. Agents **21:**364–391.
32. **Mullin, S., N. Mani, and T. H. Grossman.** 2004. Inhibition of antibiotic efflux in bacteria by the novel multidrug resistance inhibitors biricodar (VX-710) and timcodar (VX-853). Antimicrob. Agents Chemother. **48:**4171–4176.
33. **Paulsen, I. T., M. H. Brown, and R. A. Skurray.** 1996. Proton-dependent multidrug efflux systems. Microbiol. Rev. **60:**575–608.
34. **Pereda-Miranda, R., G. W. Kaatz, and S. Gibbons.** 2006. Polyacylated oligosaccharides from medicinal Mexican morning glory species as antibacterials and inhibitors of multidrug resistance in *Staphylococcus aureus*. J. Nat. Prod. **69:**406–409.
35. **Piddock, L. J., M. M. Johnson, S. Simjee, and L. Pumbwe.** 2002. Expression of efflux pump gene *pmrA* in fluoroquinolone-resistant and -susceptible clinical isolates of *Streptococcus pneumoniae*. Antimicrob. Agents Chemother. **46:**808–812.
36. **Putman, M., H. W. van Veen, and W. N. Konings.** 2000. Molecular properties of bacterial multidrug transporters. Microbiol. Mol. Biol. Rev. **64:**672–693.
37. **Ren, P. D., S. F. Pan, T. W. Dong, and S. H. Wu.** 1995. The novel reduction systems: $NaBH_4$-$SbCl_3$ or $NaBH_4$-$BiCl_3$ for conversion of nitroarenes to primary amines. Synth. Commun. **25:**3799–3803.
38. **Rottenberg, H.** 1979. The measurement of membrane potential and deltapH in cells, organelles, and vesicles. Methods Enzymol. **55:**547–569.
39. **Ryan, B. M., T. J. Dougherty, D. Beaulieu, J. Chuang, B. A. Dougherty, and J. F. Barrett.** 2001. Efflux in bacteria: what do we really know about it? Expert Opin. Investig. Drugs **10:**1409–1422.
40. **Sachs, G., and J. M. Shin.** 2004. The basis of differentiation of PPIs. Drugs Today (Barcelona) **40:**9–14.
41. **Samosorn, S., J. B. Bremner, A. Ball, and K. Lewis.** 2006. Synthesis of functionalized 2-aryl-5-nitro-*1H*-indoles and their activity as bacterial NorA efflux pump inhibitors. Bioorg. Med. Chem. **14:**857–865.
42. **Takenouchi, T., F. Tabata, Y. Iwata, H. Hanzawa, M. Sugawara, and S. Ohya.** 1996. Hydrophilicity of quinolones is not an exclusive factor for decreased activity in efflux-mediated resistant mutants of *Staphylococcus aureus*. Antimicrob. Agents Chemother. **40:**1835–1842.
43. **Van Bambeke, F., Y. Glupczynski, P. Plesiat, J. C. Pechere, and P. M. Tulkens.** 2003. Antibiotic efflux pumps in prokaryotic cells: occurrence, impact on resistance and strategies for the future of antimicrobial therapy. J. Antimicrob. Chemother. **51:**1055–1065.
44. **Wei, P., G. W. Kaatz, and R. J. Kerns.** 2004. Structural differences between paroxetine and femoxetine responsible for differential inhibition of *Staphylococcus aureus* efflux pumps. Bioorg. Med. Chem. Lett. **14:**3093–3097.
45. **Whaley, W. M., and T. R. Govindachari.** 1951. Synthesis of isoquinolines. Org. React. **6:**74–150.
46. **Yoshida, S., T. Kojima, M. Inoue, and S. Mitsuhashi.** 1991. Uptake of sparfloxacin and norfloxacin by clinical isolates of *Staphylococcus aureus*. Antimicrob. Agents Chemother. **35:**368–370.
47. **Yu, J. L., L. Grinius, and D. C. Hooper.** 2002. NorA functions as a multidrug efflux protein in both cytoplasmic membrane vesicles and reconstituted proteoliposomes. J. Bacteriol. **184:**1370–1377.
48. **Yuen, K. H., W. P. Choy, H. Y. Tan, J. W. Wong, and S. P. Yap.** 2001. Improved high performance liquid chromatographic analysis of omeprazole in human plasma. J. Pharm. Biomed. Anal. **24:**715–719.